REMARKS

Claims 9 and 10 are pending in the subject application. By this Amendment, applicant has amended claims 9 and 10 and cancelled claim 11 as withdrawn. Support for the amendments to claims 9 and 10 may be found in the specification at, inter alia, page 13, lines 17-24; and page 14, lines 3-21. Applicant maintains that these amendments raise no issue of new matter. Accordingly, claims 9 and 10 will be pending and under examination upon entry of this Amendment.

Specification

The Examiner stated that the specification is objected to for not containg the present status of parent application No. 09/234,591, which issued as U.S. Patent No. 6,706,682. The Examiner also stated that the abstract of the disclosure is objected to because the abstract contains over 150 words.

In response, applicant has amended the specification to recite the present status of the parent application. In addition, applicant has amended the abstract of the disclosure to contain less than 150 words. Applicant maintains that these amendments do not introduce new matter.

Sequence Listing

The Examiner stated that the subject application fails to comply with the requirements of 37 C.F.R. §§1.821-1.825. The Examiner

stated that applicant needs to submit a paper copy of the sequence listing, a copy of the sequence listing in computer readable form, and a statement in accordance with 37 C.F.R. §1.821(f). Furthermore, the Examiner stated that the specification discloses two sequences on page 17 and two sequences on page 18 which are not identified by sequence identifier numbers.

In response, applicant submits a paper copy of the Sequence Listing attached hereto as **Exhibit A** in compliance with the requirements of §§1.821-1.825. In addition, applicant submits herewith the Sequence Listing on the enclosed computer diskette. Moreover, applicant submits as **Exhibit B** a Statement In Compliance With 37 C.F.R. §1.821(f) certifying that the information in the computer readable form and that in the paper copy are identical, and do not introduce new matter.

Furthermore, applicant has amended the specification to include sequence identifier numbers for the sequences disclosed on pages 17 and 18 of the subject application. Applicants maintain that these amendments do not introduce new matter.

Claim Objection

The Examiner objected to claim 10 since the recited limitation "the nucleic acid comprises a vector" is interpreted to mean that the vector is any nucleic acid and therefore excludes viral vectors.

In response, but without conceding the correctness of the Examiner's objection, applicant has amended claim 10 to recite "the nucleic acid is contained within a vector." Applicant respectfully requests that the Examiner withdraw this objection.

35 U.S.C. §112, First Paragraph, Enablement

The Examiner rejected claims 9 and 10 under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the enablement requirement. The Examiner alleged that those skilled in the art would have to perform undue experimentation to make and/or use the claimed invention within its full scope, and that therefore, applicant's claims are not enabled. The Examiner asserts that vector targeting to desired tissues *in vivo* continues to be difficult.

In response, applicant respectfully traverses.

Briefly, the claimed invention provides a method for increasing or maintaining the blood supply in the penis of a subject comprising introducing a nucleic acid comprising a gene encoding a vascular endothelial growth factor into a suitable cell in the subject's penis under conditions permitting the expression of the vascular endothelial growth factor encoded by the introduced nucleic acid in the cell so as to thereby increase or maintain the blood supply in the subject's penis.

Applicant maintains that the claimed method can be practiced by one skilled in the art without undue experimentation.

As evidence of this position, applicant attaches as **Exhibit C** a copy of Burchardt et al. (2005) ("Application of Angiogenic Factors for Therapy of Erectile Dysfunction: Protein and DNA Transfer of VEGF 165 Into the Rat Penis," J. Urology, Vol. 66, No. 3, pp. 665-670 (Burchardt)).

Burchardt, which is co-authored by the applicant of this application, discloses the successful delivery of VEGF-DNA into the rat penis. Specifically, Burchardt discloses the construction of plasmids containing a human VEGF 165 isoform (page 666, right-hand column), which were then injected into the base of the corpora cavernosa of rat penises. After 28 days, the penises were harvested, and reverse transcriptase PCR was performed on the cavernosal tissue obtained from the harvested penises.

Expression of the human VEGF 165 isoform was found in the cavernosal tissue of rats injected with the plasmid containing the human VEGF 165 isoform, but not in the control rat penises (page 667, left hand column). Thus, Burchardt demonstrates that introduction of VEGF-encoding nucleic acid into corpora cavernosa tissue causes VEGF expression in that tissue.

The Examiner also asserted that the pending claims do not specify that the conditions permitting expression of VEGF permit expression of exogenous VEGF.

In response, but without conceding the correctness of the Examiner's rejection, applicant has amended claim 9 to recite

"conditions permitting the expression of the vascular endothelial growth factor encoded by the introduced nucleic acid in the cell.

In addition, the Examiner cites the reference Deonarain as evidence that the pending claims are not enabled.

In response, applicant notes that Deonarain is a review of the method of ligand-targeted receptor-mediated endocytosis as a method of gene therapy (page 54, right hand column). Deonarain does not state that successful means of gene therapy do not exist. While Deonarain does indicate that viral gene therapy has "drawbacks" it does not state that such vectors do not work. On the contrary, Deonarain states that "viral methods for gene delivery have been studied for a number of years and are effective vectors for gene transfer" (page 53, first paragraph). In addition, Deonarain states that there is a growing body of research in gene delivery using naked DNA (page 54, right hand column). Accordingly, applicant submits that Deonarain does not constitute evidence that the pending claims are not enabled.

The Examiner further cites the reference Verma *et al.* (Verma) as evidence that the pending claims are not enabled.

Verma states that the problem with gene therapy "has been an inability to deliver genes efficiently and to obtain sustained expression" (page 293, third column). However, as noted in detail above, Burchardt *et al.* discloses the successful delivery and expression of VEGF-DNA into the rat penis. Accordingly,

applicant submits that Verma does not constitute evidence that the pending claims are not enabled.

The Examiner also cites Eck *et al.* (Eck) as evidence that the pending claims are not enabled.

Eck states that several factors conceivably may be incorporated into the design of a gene transfer system so as to tailor the gene transfer to the specific requirements of the disease being treated (paragraph bridging pages 81-82). Applicant respectfully submits that these speculations on what factors may or may not conceivably be needed in a gene transfer system are irrelevant to the enablement of the pending claims since, again as noted in more detail above, Burchardt *et al.* discloses the successful delivery and expression of VEGF-DNA into the rat penis. Burchardt provides evidence that those skilled in the art can practice the claimed invention without undue experimentation and without regard to the speculations recited in Eck.

The Examiner even further cites Gorecki as evidence that the pending claims are not enabled.

Gorecki states that the most important barriers to gene therapy are "the low levels and stability of expression and immune responses to vectors and/or gene products" (page 187, abstract). However, again as noted in detail above, Burchardt *et al.* discloses the successful delivery and expression of VEGF-DNA into the rat penis. Burchardt does not disclose an immune response to the delivered and expressed VEGF-DNA. Accordingly, applicant submits that Gorecki does not constitute evidence that

the pending claims are not enabled.

The Examiner also cites the reference Bivalacqua *et al.* (Bivalacqua) as evidence that the pending claims are not enabled for treating erectile dysfunction (ED).

In response, applicant notes that the pending claims are not drawn to methods of treating erectile dysfunction. Rather, the claims are directed to maintaining and increasing the blood supply of a subject's penis. Applicant submits that Bivalacqua does not negate the enablement of the pending claims.

The Examiner also cites the reference Yancopoulos *et al.* (Yancopoulos) as evidence that the pending claims are not enabled.

Yancopoulos states that VEGF is the most critical driver of vascular formation (page 243, left hand column). In addition, Yancopoulos states that there "is no doubt that VEGF is the best-validated target for anti-angiogenesis therapy" (page 247, left hand column). Yancopoulos makes clear that VEGF is essential for maintaining or increasing blood supply to tissue. Accordingly, applicant submits that Yancopoulos does not negate enablement of the pending claims.

Finally, the Examiner cites Freed *et al.* and Banerjee *et al.* as evidence that naked viral genomes are not enabled for the claimed method.

In response, and as noted above, applicant has amended claim 10 to recite that "the nucleic acid is contained within a vector." Accordingly, the Examiner's reliance on Freed et al. and Banerjee et al. is inapposite in view of the amendments to claim 10.

In view of the above, applicant maintains that the pending claims satisfy the provisions of 35 U.S.C. §112, first paragraph.

Summary

Applicant maintains that the pending claims are in condition for allowance, and respectfully request that this application proceed to allowance.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicant's undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee, other than the enclosed fee of $225.00 for a two-month extension of time, is deemed necessary in connection with the filing of this Amendment. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

John P. White
Registration No. 28,678
Alan J. Morrison
Registration No. 37,399
Attorneys for Applicant
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, NY 10036
(212) 278-0400

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ELSEVIER

APPLICATION OF ANGIOGENIC FACTORS FOR THERAPY OF ERECTILE DYSFUNCTION: PROTEIN AND DNA TRANSFER OF VEGF 165 INTO THE RAT PENIS

MARTIN BURCHARDT, TATJANA BURCHARDT, ARISTOTELIS G. ANASTASIADIS, RALPH BUTTYAN, ALEXANDRE DE LA TAILLE, AHMAD SHABSIGH, JORGE FRANK, AND RIDWAN SHABSIGH

ABSTRACT

Objectives. To establish a laboratory animal model for vascular endothelial growth factor (VEGF) transfer in the rat penis to invent a curative therapy for erectile dysfunction (ED). Vascular insufficiency is a common pathomechanism of ED. Previous investigations have shown neovascularization of ischemic organs after gene transfer of VEGF.

Methods. For VEGF-protein transfer, osmotic pumps were connected to the renal arteries of rats. The pumps were filled with human VEGF 165 protein (n = 20) or sterile saline (n = 20). After 28 days, a VEGF serum immunoassay was performed to document successful delivery. For VEGF-DNA transfer, liposome complexes containing VEGF 165 expression vectors were injected into rat corpora cavernosa. After immunostaining, computerized image analysis was performed to quantify the percentage of area (within the corpora cavernosa) covered by smooth muscle or endothelial cells.

Results. The immunoassay of the VEGF-protein transfer showed a 10-fold greater VEGF concentration in the serum of rats carrying VEGF pumps than in the control group. In the VEGF-DNA transfer, the penes transfected with VEGF 165 vectors showed a 283-bp polymerase chain reaction product according to specific primers for human VEGF. Although statistical trends were measured in the VEGF protein-treated group, no statistically significant difference in smooth muscle or endothelial cell content was found between the control and VEGF-treated rats.

Conclusions. Our findings have established proof of principle for successful delivery of VEGF protein and VEGF-DNA transfer in the rat penis. This study was a prelude to attempt to manipulate genetically expression of angiogenic factors in insufficient erectile tissue as a curative therapy for ED. UROLOGY **66:** 665–670, 2005. © 2005 Elsevier Inc.

Erectile dysfunction (ED) is a common and undertreated disorder.[1] It is estimated that the worldwide prevalence for 2025 will be 322 million affected men.[2] A main cause of ED is vascular insufficiency.[3,4]

Despite the success and efficacy of existing therapies, the side effects, complications, and contraindications can limit their use. The "on-demand" nature of medical treatments before sexual activity is another limiting factor. In addition, that treatments are not successful in a significant subset of patients implies the need for further therapeutic improvement.

Therefore, the development of future therapeutic options should, ideally, result in a causal treatment that restores "physiologic" erectile function but has no adverse effects or contraindications. Gene therapy as a therapeutic approach has traditionally been regarded as a treatment for life-threatening disease, such as cancer or hereditary diseases. Although ED, a non-life-threatening condition, is a relatively novel area of gene therapy research, the first reports documenting "proof of concept" were published in the late 1990s.[5,6]

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen and an angiogenic growth factor.[7] It is produced by vascular

From the Department of Urology, Medizinische Hochschule Hannover, Hannover, Germany; and Departments of Urology and Dermatology, Columbia University College of Physicians and Surgeons, New York, New York

Reprint requests: Ridwan Shabsigh, M.D., Department of Urology, Columbia-Presbyterian Medical Center, Herbert Irving Pavilion, 11th Floor, 161 Fort Washington Avenue, New York, NY 10032. E-mail: rshabsigh@urology.columbia.edu

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smooth muscle, endothelial, and inflammatory cells and has been shown to increase the production of nitric oxide, which, in turn, may improve endothelial function.[8,9] VEGF has been shown to significantly improve blood flow in vivo in chronic ischemic disorders, including a rabbit ischemic hind limb model[10,11] and a porcine myocardial ischemia model.[12]

Angiogenesis and improved vasoactive function are thought to be responsible for these effects on blood flow. Recently, studies from two institutions have reported enthusiastic results—mainly functionally measured by cavernosometry—regarding outcome of arteriogenic, venogenic, and nerval forms of ED using direct intracavernosal injection of recombinant VEGF protein or adenoviral VEGF gene containing plasmids in the rat penis.[13–18] These positive results, based only on a single-agent strategy (ie, VEGF application), were surprising, since other reports have described the process of angiogenic remodeling as a complex genetic evolution.[19,20]

Therefore, this study addressed the question of whether VEGF by itself, administered using two different approaches, has an impact on smooth muscle and/or endothelial cell density in corporal tissues. As a prelude to these experiments, we first described the different and predominant VEGF isoforms in the rat and human penes.[21] Here, we report, for the first time, the effects of systemic application of VEGF 165 protein and VEGF-DNA transfer using microinjection of plasmids into the corpora cavernosa of rats.

MATERIAL AND METHODS

Laboratory Animals

Forty age-matched male Sprague-Dawley rats (10 weeks old, Camm Laboratories, Camden, NJ) were obtained for each delivery model (protein transfer plus control, n = 20 + 20; gene transfer plus control, n = 20 + 20) under an Institutional Animal Care and Use Committee (IACUC)-approved protocol and were maintained on a 12-hour daylight cycle with food and water available ad libitum.

Pump Implantation for VEGF Protein Transfer

Osmotic pumps with a fluid capacity of 200 μL (Model 2002, Alzet, Mountain View, Calif), were filled with sterile saline alone (control group) or 1 μg/mL human VEGF 165 protein (VEGF group; R&D Systems, Minneapolis, Minn). The delivery rate was 0.25 μL/hr for a 28-day period, as was used successfully in previous studies.[12] Also, 2000 U heparin (Sigma Chemical, St. Louis, Mo) were added to each pump to ensure permeability of the catheter.

The rats were anesthetized with sodium pentobarbital, and the left renal artery was exposed through a transperitoneal approach. A small polyethylene catheter (Fisher Scientific, Santa Clara, Calif), which was connected to the intraperitoneally implanted osmotic pump, was inserted into the left renal artery for continuous infusion of VEGF protein. Subsequently, the catheter was tied into the renal artery by two silk 4-0 sutures.

VEGF Immunoassay

The rats were killed 28 days after pump implantation. Immediately before overdose pentobarbital administration, blood was drawn from the abdominal aorta. The concentration of serum VEGF was measured by an immunoassay (Quantikine M, R&D Systems) according to the manufacturer's instructions.

VEGF Plasmid Constructs

For RNA extraction, total RNA was isolated from tissue powder of the human lung (Tissue Bank, Department of Pathology, Columbia University, New York, NY) using an RNAzol extraction procedure (Tel-Test, Friendswoods, Tex), following the manufacturer's protocol. The RNA concentration was determined by spectrophotometry at 260 nm. To ensure that the RNA was not degraded, each sample was analyzed by electrophoresis.

Reverse Transcriptase-Polymerase Chain Reaction to Amplify VEGF cDNA

First-strand cDNA synthesis was performed as previously published by Burchardt *et al.*[21] Polymerase chain reaction (PCR) techniques were used to amplify human VEGF 165 isoform. Specific primers for amplification of all known VEGF splice variants were designed as follows: 5′-GGT CGG GCC TCC GAA TTC ATG AAC TTT CTG CT-3′ and 5′-GGG AGG ATC CTT CCT CCT GCC CTC ACC G-3′.

The PCR reaction, visualization of DNA bands, and cloning of VEGF plasmid constructs has been previously described.[21]

VEGF-DNA Transfer: Microinjection of Plasmids into Rat Corpora Cavernosa

An incision was made through the perineum of the anesthetized rats. Then, 100 μg VEGF 165/pIRES^{neo} plasmid (or pIRES^{neo} vector as a negative control) in 100 μL phosphate-buffered saline were mixed with 100 μL Lipofectamine (Gibco BRL, Grand Island, NY). The resulting liposome complexes were injected into the base of the corpora cavernosa. After 28 days, the rats were killed by a lethal overdose of sodium pentobarbital. The penes were harvested as described previously,[22] and the delivery of the microinjection was proven by PCR of the penile DNA transfected with VEGF 165/pIRES^{neo} plasmid.

Transfection Efficiency

Gene expression was evaluated at the mRNA level by reverse transcriptase (RT)-PCR of cavernosal tissue obtained from rats treated with VEGF gene transfer. Specific primers selected from regions not conserved among different species were designed to amplify a 283-bp sequence of human VEGF 165: upstream primer (human VEGF 165$^{position\ 154–170}$): GAG GGC AGA ATC ATC AC; downstream primer (human VEGF 165$^{position\ +23–+37}$): TTT GTT GTG CTG TAG. The PCR amplification was performed for 35 cycles, starting with 7 minutes at 94°C, denaturation for 45 seconds at 94°C, annealing for 45 seconds at 60°C, extension for 45 seconds at 72°C, and ending with 15 minutes at 72°C. The PCR product was analyzed by electrophoresis on a 2% agarose gel that was subsequently stained with ethidium bromide for visualization of DNA bands.

Immunohistochemistry and Computerized Image Analysis

Immunohistochemistry and computerized image analysis for corporal endothelial and smooth muscle cells were performed, as previously described by the authors.[22] The antibod-

TABLE I. ***Mean area of smooth muscle cells and endothelial cells in rats treated with VEGF gene and protein transfer vs. control groups***

	Protein Transfer		DNA Transfer	
	VEGF (n = 20)	Control (n = 20)	VEGF (n = 20)	Control (n = 20)
Smooth muscle cell area	16.89 ± 2.88*	14.15 ± 2.22	15.06 ± 2.12†	13.7 ± 2.57
Endothelial cell area	7.12 ± 1.22‡	5.99 ± 1.31	6.58 ± 1.43§	5.41 ± 1.15

KEY: VEGF = vascular endothelial growth factor.
Data presented as mean percentage ± standard deviation.
* P = 0.0641 vs. protein control.
† P = 0.3864 vs. DNA control.
‡ P = 0.0742 vs. protein control.
§ P = 0.0986 vs. DNA control.

ies used to identify endothelial cells were primary mouse monoclonal antibody anti-rat CD31 (Serotec, Oxford, England; dilution 1:1000) and monoclonal anti-alpha-smooth muscle actin antibody (Sigma Chemical; 1:50).

Statistical Analysis

Statistical analysis was performed through the Department of Biostatistics of Columbia University. The data were analyzed using a statistical analysis program (Statistical Analysis Systems, version 6.12, SAS Institute, Cary, NC), and the data between groups were compared by analysis of variance on the square root of the Arcsine of the percentage of area of the endothelial or smooth muscle cells in each group.

RESULTS

At 28 days after implantation of the osmotic pumps, blood was drawn from the aorta of the rats. A 10-fold greater mean serum concentration of VEGF protein (1423 ± 67 pg/mL) was measured by a quantitative VEGF immunoassay (Quantikine M, R&D Systems) in the group of animals treated with pumps containing VEGF protein versus the group treated with pumps containing sterile saline (150 ± 32 pg/mL, $P < 0.05$).

Fixed penile tissues from control, VEGF protein-treated, and VEGF gene-treated rats were sectioned and immunostained for alpha-smooth muscle actin (to identify smooth muscle cells) and anti-CD31 (to identify endothelial cells). The cavernosal regions in these immunohistochemically stained specimens were outlined using a computerized imaging program and the percentage of area (within the cavernosum) occupied by red-staining smooth muscle cells or brown-staining endothelial cells was quantified by the computer. Five separate images per animal were analyzed by this method (Table I). Borderline *P* values were measured for an increase of both endothelial ($P = 0.0742$) and smooth muscle ($P = 0.0641$) cell expression after VEGF protein transfer; the *P* value was 0.0986 for the increase in endothelial cells after VEGF-DNA transfer. However, none of the *P* values were significant for treatment with either VEGF protein or VEGF-DNA transfer. Furthermore, no trend was discovered to show an increase in smooth muscle cell area after VEGF gene therapy ($P = 0.3864$).

RT-PCR of VEGF cDNA and consecutive gel electrophoresis revealed a 283-bp band in rat penes treated with VEGF DNA. This 283-bp band corresponded to the size of human VEGF 165, amplified by primers specific for human VEGF. These oligonucleotide primers were selected from human regions not conserved among the different species to ensure specificity and avoid amplification of endogenous rat VEGF. No band of corresponding size was amplified in the control rats (Fig. 1). This finding serves as a proof of successful VEGF expression in rat corpora cavernosa after cDNA microinjection of VEGF plasmid.

COMMENT

Although VEGF is one of the most intensively studied angiogenic growth factors, only a few research groups have studied its role in penile physiology. This observation is surprising, because this endothelial cell-specific mitogen has demonstrated angiogenic properties in vivo. Administration of VEGF was capable of significantly improving blood flow to ischemic myocardium,[11,12] accelerating reendothelialization in balloon-injured rat carotid arteries,[23] and improving blood pressure, as well as arterial collateralization, in a rabbit model of hind limb ischemia.[10] Until now, experiments regarding VEGF application as a potentially curative treatment for ED have only been published by two groups, both of whom measured positive effects in cavernosometry, isometric tension studies, or immunohistochemistry after VEGF administration.[14,15]

During our first approach to the use of VEGF as a potentially curative treatment for ED, we identified VEGF 165 as the predominant isoform of the corpora cavernosa.[21] Subsequently, we identified a novel VEGF splice variant in the penis.[24] Several reports examining the effect of intracavernosal VEGF injections followed. Using the rabbit model, Henry *et al.*[13] investigated whether VEGF could be

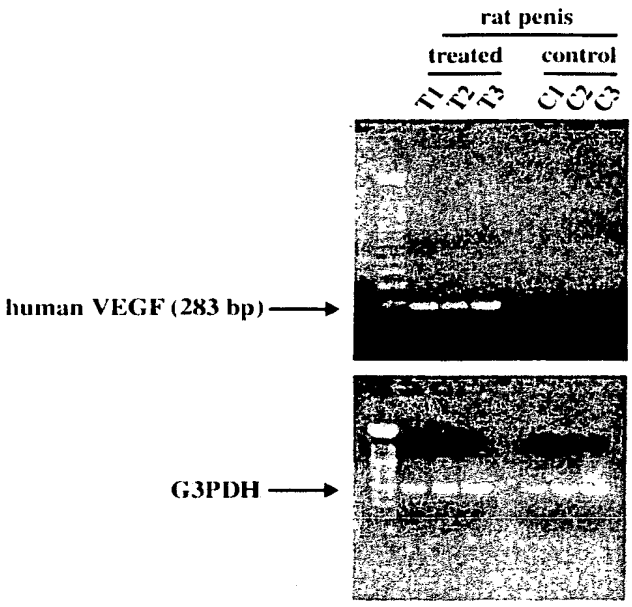

FIGURE 1. *Gel electrophoresis of VEGF gene expression by RT-PCR in rat corporal tissue transfected with human VEGF 165 or control plasmid. Penile tissues of 18 rats treated with VEGF gene transfer and penile tissues of 18 control rats were randomly divided into three groups (control group = C1 to C3; VEGF group = T1 to T3) and analyzed for human VEGF 165 gene expression. Gel electrophoresis revealed 283-bp band in penes of rats treated with VEGF DNA (T1 to T3). This 283-bp band corresponded to size of human VEGF 165, amplified by primers specific for human VEGF. No band of corresponding size (283 bp) was amplified in control rats (C1 to C3). This finding serves as a proof of successful VEGF expression in rat corpora cavernosa after cDNA microinjection of VEGF plasmid. G3PDH expression served as positive control.*

used to protect endothelial-dependent cavernosal relaxation from atherosclerotic injury induced by a hypercholesterolemic diet. Isometric tension studies were performed on isolated corpora cavernosa strips after weekly penile VEGF or saline injections. The hypercholesterolemic rabbits receiving VEGF had a significantly greater maximal percentage of relaxation to acetylcholine compared with the hypercholesterolemic rabbits that received normal saline, suggesting a protection of the corporal endothelium from hypercholesterolemia-induced injury.

Another in vivo study investigated the effect of a single intracavernous injection of VEGF in a rat model of traumatic arteriogenic ED.[25] At weeks 1, 2, and 6 after the procedure, functional testing of erectile function using electrostimulation of the cavernous nerves and intracavernous pressure measurement was performed. VEGF treatment resulted in significant recovery of erectile function after 6 weeks.

A potential gene therapy approach from the same group targeted vasculogenic and neurogenic ED by intracavernous injection of VEGF and adeno-associated virus-mediated, brain-derived neurotrophic factor in hypercholesterolemic rats. In addition to severe atrophy of the axons, the destruction of smooth muscle and endothelial cells was significantly greater in the control group than in the VEGF-treated samples.[18]

In contrast to the previously mentioned reports, in which VEGF was used only for single-shot intracavernous injections, the present study included systemic treatment (ie, continuous intra-arterial VEGF protein administration with an osmotic pump) for a 4-week period, a time frame that was successfully applied in other ischemic diseases.[12] In a second, localized approach for VEGF transfer, human VEGF DNA was directly injected into the corpora cavernosa of the rats. The effective delivery of human VEGF to the corpora cavernosa of the rat penis was proven by RT-PCR using custom-designed primers (Fig. 1). These two different models of VEGF treatment were used to provide data for comparison of a continuous systemic versus direct application in the same experimental setting.

For the first time, it could be demonstrated that VEGF was present in significantly greater serum concentrations in the VEGF-treated rats than in the controls. In addition, the successful corporal transfer of VEGF DNA was proven by PCR. Despite a statistically significant trend in the evaluation of the contents of smooth muscle and endothelial cells, however, we did not observe a statistically significant difference between VEGF-treated and control animals. These results were somehow not concordant with those of previous studies, which have demonstrated a morphologic, molecular, and functional response to intracavernosal VEGF administration.[15–18] Animal age and the interval after VEGF treatment were comparable to those of previous studies, and the dosage of VEGF was sufficient when compared with these reports. However, our results are in concordance with numerous publications focusing on the complexity of angiogenesis, hereby considering VEGF's tendency to produce leaky vessels and hemorrhage.[20] Furthermore, despite its requisite role in vascular formation, newer insights in vasculogenesis let us assume that VEGF must act in concert with many other important factors (eg, angiopoietins and Eph receptor kinases). Although VEGF has been shown to be a potent and critical vascular regulator, its dosage must be exquisitely regulated spatially, temporally, and quantitatively.[19] In addition, VEGF does not seem to have a continuous maintenance function in the adult vasculature, a phenomenon that explains the observation of the decrease of VEGF effects with time and that may be a disadvantage of single administration.

For these reasons, if one looks closely at the multifactorial process of vascular formation, random delivery of a single agent to treat ischemic disease appears debatable. Recent failures of large, well-controlled clinical trials of cardiac ischemia using delivery of single agents (either VEGF or fibroblast growth factor) raises the question of why these trials failed despite claims of success in animal studies and earlier, smaller, and uncontrolled human trials.[26,27] This phenomenon may have been a result of the failure of animal models to model the human disease correctly, as well as the need for blinded approaches in both animal and human studies to overcome investigator bias when measuring subjective endpoints.[19] As an arguably favorable observation for the previously mentioned studies showing a statistically relevant gain in intracavernous pressure after VEGF application, one may recognize the statistical trends in both smooth muscle and endothelial cell staining after protein transfer in our experiments (Table 1). Furthermore, our results reflect measurements in normal animals, in contrast to previous data. With regard to possible VEGF-promoted carcinogenesis as an unwanted side effect, one may hope that the lack of proliferative activity in normal vasculature truly reflects the biology of VEGF action and that neovascularization is only promoted in animal models of vasculogenic ED.

Many critical factors are involved in the physiologic regulation of blood vessel formation, and the actions of these molecular players must be carefully orchestrated. For these reasons, additional studies are necessary to investigate the effects of angiogenic factors in penile pathophysiology before an effective angiotherapeutic possibility for ED can be pursued.

CONCLUSIONS

Reports about VEGF-promoted effects on erectile function are scant and based only on nonblinded, uncontrolled results from a few institutions. In the present experiments, we studied the effects of a unique systemic and local administration of VEGF in the rat model. However, neither route of administration of VEGF was able to demonstrate a statistically relevant increase in smooth muscle and endothelial cell content in the corpora cavernosa. Because the previously described angiogenic benefit of VEGF administration to the penis was not obvious and reproducible in this study, additional, blinded, and controlled experiments are necessary. Before human trials for a benign disease such as ED can be conducted, future studies need to determine the adequate VEGF dosage, time point, and route of administration, as well as the possible addition of other angiogenic factors, for improvement of ED therapy.

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